MICROBIOLOGICAL REVIEWS, March 1994, p. 56–70 Vol. 58, No. 1
0146-0749/94/$04.00+0

Control of Meiotic Gene Expression in *Saccharomyces cerevisiae*

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INTRODUCTION

Cells of the budding yeast *Saccharomyces cerevisiae* produce mitotic daughters whenever nutrients are plentiful. However, starvation causes cell growth and mitotic division to cease. One type of cell, the **a**/α diploid cell, then initiates a sporulation program that leads through meiosis to spore formation. The other two types of cells, **a** and α haploid cells, become arrested in a G_1 phase of the mitotic cell cycle. The focus of this article is the regulatory system that permits **a**/α cells to sporulate. Other reviews have discussed regulation of meiosis in *S. cerevisiae* (38, 57, 59, 90) and *Schizosaccharomyces pombe* (64, 116) and meiotic recombination (1a, 48, 81, 82).

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Two nutritional conditions are required for sporulation. One is limitation for an essential nutrient. Nitrogen limitation causes efficient sporulation and is generally used in the laboratory to induce sporulation. However, limitation for carbon, phosphate, sulfate, guanine, methionine, and other compounds can also cause sporulation (26, 108). The other condition is absence of a fermentable carbon source, such as glucose. Sporulation medium typically contains acetate, although pyruvate and ethanol are also suitable (26). The carbon source apparently governs the decision between pseudohyphal growth and sporulation: nitrogen limitation in the presence of glucose leads to pseudohyphal growth (29); nitrogen limitation in the absence of glucose leads to sporulation.

The signal that specifies cell type comes from alleles of the mating type locus, or *MAT* (34). Haploid **a** and α cells have *MAT***a** or *MAT*α alleles, respectively, while diploid **a**/α cells have both *MAT***a** and *MAT*α alleles. The ability to sporulate

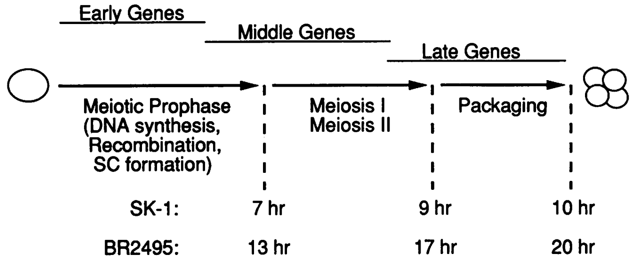

FIG. 1. Time course of meiotic events. The main phases of the sporulation program are indicated in relation to the times at which early, middle, and late genes are expressed. The time after starvation at which each phase occurs is indicated for SK-1-derived strains (71) and BR2495-derived strains (65). The relative times of early, middle, and late meiotic gene expression are from references 114, 57, and 65. SC, synaptonemal complex.

requires the expression of both *MAT***a** and *MAT*α. Thus, diploid **a**/**a** and α/α cells, which have two *MAT***a** or two *MAT*α alleles, are unable to sporulate (83). *MAT***a** and *MAT*α specify **a**1 and α2, respectively, which are subunits of the transcriptional repressor **a**1-α2 (19, 31). **a**1-α2 is ultimately responsible for all known **a**/α cell properties. In this article, I will refer to cells that lack **a**1 or α2 as non-**a**/α cells. Because **a**1-α2 expression is generally restricted to diploid cells, it indicates that cells have the necessary number of chromosomes for successful meiotic divisions.

The landmark events of sporulation have been established by comparison of starved **a**/α cells and non-**a**/α cells (25, 71) (Fig. 1). Cells enter meiosis through the meiotic prophase, which includes a round of DNA synthesis and events associated with recombination: chromosomes condense, transient double-stranded chromosome breaks occur, and gene convertants and recombinants appear. In addition, the synaptonemal complex forms. (See reference 71 for a careful kinetic study.) Cells then go through the meiosis I (reductional) and meiosis II (equational) divisions. Finally, spore walls form through deposition of spore coat materials within a membrane outgrowth near the spindle poles (6, 10).

A set of genes referred to as meiotic genes or sporulation-specific genes display much higher RNA levels in sporulating cells than in either vegetative cells or starved, non-**a**/α cells (Table 1 and references therein). These genes have been identified through two general approaches. One is based on gene function: mutants with specific meiotic defects (such as recombination, spore packaging, or reductional division) were identified, and studies of the corresponding genes revealed that their transcripts accumulated only in sporulating cells. Not all mutations that cause meiotic defects lie in meiotic genes, however. For example, *spo7* mutations block sporulation, but *SPO7* is expressed in vegetative cells (115). The second approach is based on gene expression: genes expressed preferentially in starved **a**/α cells were identified by differential hybridization (2, 12, 30, 73) or *lacZ* fusion protein expression screens (13). Surprisingly, the bulk of the genes identified by this second approach have turned out to be dispensable for the sporulation program.

Meiotic genes have been divided into three classes—early, middle, and late—based on their time of expression (12, 57, 73, 114). Early genes are expressed at the beginning of meiotic prophase; middle genes are expressed later in prophase; and late genes are expressed around the time of meiotic divisions and spore packaging (Fig. 1). The restructuring of the cell during sporulation leads to differential compartmentation of later transcripts, in the ascal versus spore cytoplasm (49) and even in one spore but not another (6). These temporal groupings are only approximate for two reasons. First, some genes are expressed at unique times. *DIT1* and *DIT2*, for example, are expressed after middle genes but before late genes (6). Second, the kinetics and synchrony of sporulation vary from strain to strain. Thus, the groupings in Table 1, particularly for middle and late genes, should be considered provisional.

Sporulating cells express many genes that are expressed at similar levels under other circumstances. For example, several heat shock genes are expressed at elevated levels after starvation of both **a**/α and non-**a**/α cells (50). Transcripts of many DNA synthesis and repair genes accumulate to elevated levels during meiotic prophase, as they do during mitotic S phase or after DNA damage (40–42, 56, 78, 84). It is noteworthy that increased RNA levels may not cause a corresponding increase in protein product levels (78). Some of these genes are clearly required for sporulation, while others are not (9, 25, 75, 89).

STRUCTURE OF MEIOTIC PROMOTERS

The regulatory sequences of three early meiotic genes and two later genes have been analyzed in some detail. These studies, combined with studies on the sequences of other meiotic genes, indicate that many early genes have common regulatory sequences. In addition, later genes may share a distinct regulatory sequence. Thus, the temporal sequence of meiotic gene expression may reflect the order in which classes of promoters are activated.

Early Meiotic Genes

Functional analysis of 5′ regions of the early genes *SPO13*, *HOP1*, and *IME2* suggests four broad conclusions (3, 8, 109). First, these genes contain a site, URS1, that is a repression site in promoters of nonmeiotic genes (102). Second, URS1 represses early meiotic promoters in nonmeiotic cells but stimulates these promoters in meiotic cells. Third, a nearby site often participates along with URS1 in stimulating meiotic gene expression. Finally, many early meiotic promoters have regulatory sequences that are very close to minimal promoter sequences (that is, the TATA and RNA start sites).

Analysis of the *SPO13* regulatory region first implicated a URS1 site in meiosis-specific expression (8). A *spo13-lacZ* fusion that included only *SPO13* sequences between −140 and +45 displayed meiosis-specific expression; the fusion was silent in growing **a**/α cells and expressed in starved **a**/α cells. No expression was detected in growing or starved non-**a**/α cells. A point mutation in the URS1 site (at −92) caused a sixfold decrease in *spo13-lacZ* expression in meiotic cells. Decreased expression was also observed with a *spo13-lacZ* fusion that extended only to −80 and therefore lacked the URS1 site. These findings, together with the observation that many early meiotic genes have URS1 sites (8), indicated that URS1 may have a positive role in early meiotic gene expression.

Both the URS1 point mutation and the deletion to −80 also caused slightly elevated *spo13-lacZ* expression in nonmeiotic cells. Buckingham et al. (8) pointed out that expression may have resulted from adventitious vector upstream activation sequences (UASs) or promoters. However, this observation suggested that the URS1 site in the *SPO13* promoter is a negative site in nonmeiotic cells (8).

TABLE 1. Sporulation-specific genes

Time of expression[a]	Gene	Isolation[b]	Function[c]	Regulatory site(s)[d]			Reference
				URS1	UAS_H	T_4C site	
Early	*DMC1*	Expression	Recombination	−137, TgGGCGGCT	−168, TGTGgAGaG	−195, TTTTCTTCG	2
	HOP1	Function	Recombination	−173, TgGGCGGCT	−198, TGTGAAGTG		35
	IME1[e]	Function	Transcription				45
	IME2, *SME1*	Function	Transcription	−552, aCGGCGGCT; −457, TCGGCGGCT		−581, TTTTCTCCG; −478, TTTTCCCtG	118
	IME4	Function	Transcription	—	—	—	86
	MEI4	Function	Recombination	−98, TgGGCGGCT			65
	MEK1, *MRE4*	Function	Recombination	−136, TCGGCGGCT; −150, atGGCGGCT		−177, TTTTCCCCG	53, 80
	MER1	Function	Splicing	−111, TCGGCGGCT	−270, aGTGAAaTa		23
	REC102	Function	Recombination	−397, TCaGCGGtG	−443, TGTaAAGTG	−437, TTTTCGCCt	14, 60
	REC104	Function	Recombination	−93, TtGGCGGCT	−139, ccaGtAGTG	−83, TTTTCCGCt	27, 36
	REC114	Function	Recombination	−90, TgGGCGGCT	−291, TGTGAttTt		76
	RED1	Function	Recombination	−166, TCaGCGGCT		−206, TTTTCAACG	105
	RIM4	Function	*IME2* expression	−394, TgGGCGGCT; −336, TCGGCGGCT		−121, TTTTCTTCG	101
	SPO11	Function	Recombination	+163, TtGGCGGCT	−252, TGTGtAGTG		1
	SPO13	Function	Meiosis I division	−96, TCGGCGGCT			114
	SPO16	Function	Sporulation efficiency	−90, TgGGCGGCT	−198, TGTGAtGTa		58
	ZIP1	Function	Synaptonemal complex formation	−22, TCGGCGGCT			104
Middle	*SIT2*	Expression	Nonessential; unknown	—	—	—	30
	SIT3	Expression	Nonessential; unknown	—	—	—	30
	SIT4	Expression	Nonessential; unknown	—	—	—	30
	SPO12	Function	Meiosis I division	−220, agGGCatCT	−345, TGTGgAaaa		58
	SPS1	Expression	Postmeiotic events	—	—	—	74
	SPS2	Expression	Nonessential; unknown				74
	SPS3	Expression	Unknown	—	—	—	74
	SPS4	Expression	Unknown				28
Mid-late	*DIT1*	Function	Spore wall formation				6
	DIT2	Function	Spore wall formation				6
Late	*SGA1*	Homology	Nonessential; glucoamylase				117
	SPR1	Expression	Nonessential; β-glucanase				12, 69
	SPR2	Expression	Nonessential; unknown	—	—	—	12, 77
	SPR3	Expression	Nonessential; unknown	—	—	—	12
	SPS100	Expression	Spore wall maturation				52
	SPS101	Expression	Unknown	—	—	—	52

[a] Conclusions about expression class are generally those drawn in primary references. There is some ambiguity in comparing middle and late genes reported from different laboratories.

[b] Isolation refers to the basis for initial identification of the gene. Function, identified through mutant phenotype; expression, identified by expression pattern of transcript; homology, identified by cross-hybridization with a functionally related gene.

[c] Genes in which insertions or deletions cause no sporulation defect are designated nonessential.

[d] Locations of URS1, UAS_H, and T_4C sites are from references 3, 8, and 109 and from sequences reported for individual genes. Nucleotide coordinates refer to the 5′-most residue of each sequence. Some sites are reported in inverse orientation to simplify comparison. Consensus sequences: URS1, TCGGCGGCT (8); UAS_H, TGTGAAGTG (109); T_4C site, TTTTCXXCG (3). Bases that deviate from each consensus are shown in lowercase letters. —, no sequence has been reported.

[e] *IME1* is not strictly a meiosis-specific gene (47, 87), as described in the text.

Studies of *HOP1* have also pointed to both positive and negative roles for URS1 (109). *HOP1* sequences between −207 and +18 direct meiosis-specific expression of an *hop1-lacZ* fusion. Destruction of a URS1 site at −173 through a multisite mutation causes midlevel constitutivity: expression is elevated in nonmeiotic cells (growing **a**/α cells) and reduced in meiotic cells. Although the URS1 mutation reduced meiotic cell β-galactosidase levels only a few fold, this activity may have accumulated during vegetative growth before transfer into sporulation medium. Nonmeiotic expression of a *hop1-lacZ* fusion lacking URS1 was clearly not due to vector sequences, because expression was abolished by a second multisite mutation within *HOP1* 5′ sequences. This second mutation defined an element called UAS_H, which has UAS activity in nonmeiotic cells when separated from the URS1 site. Therefore, at *HOP1*, URS1 blocks UAS_H activity in nonmeiotic cells. In addition, URS1 stimulates *HOP1* expression in meiotic cells.

Both *SPO13* and *HOP1* have regulatory regions that are very close to their initiation codons (Table 1). For *SPO13*, RNA start sites have been mapped in the −49 to −10 interval, only 40 to 80 bp from the URS1 site (114). Yeast genes generally have regulatory sequences that lie upstream of, and are separable from, TATA and RNA start sites (98). Many genes have UASs that can confer regulated expression when fused to a heterologous minimal promoter, consisting only of TATA and RNA start sites. However, no meiosis-specific regulatory region has been separated from the minimal promoter elements of *SPO13* and *HOP1*. One explanation is that these promoters simply pose some technical difficulty (e.g., meiotic regulatory sequences and minimal promoter sequences may overlap). A second possibility is that expression of these genes is achieved by an unusual mechanism (e.g., a single site may serve as both an activation sequence and a TATA sequence).

The *IME2* gene has a structure more typical of yeast genes. Deletions that abolish expression affect sequences between −584 and −442 (3). Although *IME2* has a long (ca. 300-base) untranslated leader (20), these deletions lie quite far from the RNA start site. This interval has the properties of a meiosis-specific UAS, because placing it upstream of a minimal promoter causes meiosis-specific expression of the reporter gene. In these studies, meiosis-specific expression was assessed by dependence of expression on an activator of meiosis, *IME1* (see below). Further subcloning revealed that this region contains two separable *IME1*-dependent UAS regions, a stronger upstream UAS and a weaker downstream UAS. Thus, *IME2* has a meiosis-specific upstream regulatory region that is separable from other promoter elements.

Mutational analysis of the strong *IME2* UAS indicates that here, as in the *SPO13* and *HOP1* promoters, a URS1 site plays both positive and negative roles (3). A second site, called a T_4C site, also contributes to UAS activity, much like the *HOP1* promoter. URS1 mutations caused midlevel constitutive (*IME1*-independent) expression, whereas T_4C site mutations simply reduced UAS activity without relieving *IME1* dependence. These observations led to the suggestion that the URS1 and T_4C sites have different roles in UAS activity: URS1 is required to confer *IME1* dependence, whereas the T_4C site adjusts the overall expression level (3).

URS1, UAS_H, and T_4C sites are found in the regulatory regions of many early meiotic genes (Table 1). The URS1 site generally lies within 200 bp of the initiation codon; *IME2*, *REC102*, and *RIM4* are the exceptions. A subset of genes (*HOP1*, *MER1*, *REC102*, *SPO11*, and *SPO16*) also have a nearby UAS_H site; a different subset (*IME2*, *MEK1*/*MRE4*, *RED1*, and *RIM4*) have a nearby T_4C site. The UAS_H or T_4C site generally lies upstream of URS1. Some unusual cases include *DMC1*, which has URS1, UAS_H, and T_4C sites; *SPO13* and *MEI4*, which have URS1 sites without discernible UAS_H or T_4C sites; and *SPO11*, which has a URS1 site within the coding region. *MEK1*/*MRE4* has been cloned and sequenced by two groups, which found one and two URS1 sites, respectively. *IME2* has two pairs of URS1 and T_4C sites; one pair lies within each of the *IME2* UASs. The weaker *IME2* UAS has a poorer match to the T_4C site consensus. The middle gene *SPO12* was reported to have both URS1 and UAS_H homology (109), but both sequences are very poor matches to each consensus. Thus, URS1 and an accompanying UAS_H or T_4C site are found near many early meiotic genes.

Two simple models can account for the roles of each site at early meiotic regulatory regions. One possibility is that meiosis-specific expression results only from a unique interaction between URS1 and an accompanying site. Each site in isolation would have properties unrelated to meiotic expression. Meiotic genes without UAS_H or T_4C site homology presumably have a similar type of site that has yet to be identified. The second possibility is that the URS1 site is a meiotic on/off switch that specifies which genes may be activated early in meiosis. The accompanying UAS_H or T_4C site serves as a gain control that determines the overall level of expression. Independent on/off and gain controls may be useful for a large family of genes with a wide range of expression levels. Studies of the individual sites and the relevant regulatory proteins will be necessary to distinguish these and other more complicated models.

The *IME1* gene is expressed at high levels early in meiosis, yet it has no recognizable URS1 site. *IME1* may be expressed a little earlier than most early meiotic genes (47, 67) and therefore may belong to a distinct expression class. Arguments based on function suggest that *IME1* expression should precede that of other early genes (see below). However, *IME1* is expressed at high levels after heat shock (87) and at the end of exponential growth (47). These conditions do not lead to meiosis, so *IME1* is not strictly a meiosis-specific gene. *IME1* may have an unusual regulatory region because its expression pattern is different from that of most early meiotic genes. However, these observations leave open the possibility that URS1-dependent expression is only one of the ways that early meiosis-specific expression is achieved.

Middle and Late Meiotic Genes

Expression of the late sporulation-specific gene *SGA1* also depends on two sequence elements (48). One element functions in a heterologous promoter as a UAS. Expression of the heterologous gene containing the *SGA1* UAS is blocked by the presence of either glucose or ammonia. The second element, called the negative regulatory element (NRE), functions in a heterologous promoter as a negative site. Expression of the NRE-containing hybrid promoter is restricted to **a**/α cells and depends on starvation and on the positive meiotic regulators *IME1* and *IME2*. A 17-bp segment of the NRE (AGGGTC CTTTTTTGGTT) includes 14 identities to a 5′ segment of a middle sporulation-specific gene, *SPS4*. Note that expression of *SPS4* and *SGA1* has not been monitored in the same experiment, so the genes may belong to the same temporal class. These observations indicate that expression of some middle and late meiotic genes may depend on relief of repression through the NRE (48).

Analysis of the late gene *SPR2* supports the importance of an NRE-like sequence but suggests a positive role for the site

(77). Deletion analysis indicates that a positive site lies near −240, and a 31-bp segment from this region has UAS activity in starved **a**/α cells but not in growing **a**/α cells. The UAS becomes active at 6 h after starvation (in SK-1 strains), which coincides with the time that *SPR2* is expressed. The UAS includes a 16-bp match to the *SGA1* NRE, including 9 bp shared between *SGA1* and *SPS4*. The most parsimonious model (77) is that the *SPR2* UAS includes both a positive site and an adjacent or overlapping negative site, the latter corresponding to the *SGA1* NRE.

MEIOTIC REGULATORY GENES

Many known genes influence meiotic gene expression. Understanding the function of each relies on understanding its relationship with upstream and downstream regulators. For that reason, this section begins with a summary of the identification and properties of many key regulators. Several groups of genes have been identified through similar strategies. Increased dosage of *IME* (inducer of meiosis) genes stimulates meiosis in non-**a**/α cells. Mutations in *UME* (unscheduled meiotic gene expression) genes permit *SPO13* promoter activity in vegetative, non-**a**/α cells. Mutations in *RIM* (regulator of inducer of meiosis) genes prevent expression of an *ime2-lacZ* fusion gene. Mutations affecting cyclic AMP (cAMP)-dependent protein kinase activity affect meiotic gene expression, but these genes are not listed individually and will be discussed only briefly (see reference 7 for a more thorough review of this topic).

IME1. *IME1* was identified as a multicopy genomic clone that permits non-**a**/α diploids to sporulate (45). An *ime1* disruption prevents expression of almost all meiotic genes and all tested meiotic events (23, 45, 48, 67, 92, 109, 118). *IME1* has no informative homologies (94).

IME2. *IME2* (also called *SME1*, for start of meiosis) was identified as a multicopy genomic clone that permits **a**/α cells expressing an inhibitor of meiosis, *RME1*, to undergo recombination (92). *IME2* was also isolated as a multicopy clone that permits sporulation in the presence of a nitrogen source (118). An *ime2* disruption mutant shows reduced or delayed recombination and DNA synthesis and reduced expression of middle meiotic genes (92, 118). Ime2 is a protein kinase homolog (118).

IME4. *IME4* was identified as a clone that enhances *RES1-1*-dependent *spr3-lacZ* expression in non-**a**/α cells (86). An *ime4* disruption reduces or abolishes *IME1* and *IME2* expression and sporulation.

MCK1. *MCK1* (meiosis and centromere regulatory kinase) was identified as a multicopy genomic clone that permits **a**/α cells expressing *RME1* to undergo meiotic recombination (70). *MCK1* was independently identified as a multicopy suppressor of mitotic chromosome missegregation arising from centromere mutations (88). An *mck1* disruption reduces the rate and efficiency of meiotic gene expression and meiosis, causes accumulation of immature asci, and causes defects in mitotic centromere behavior (70, 88). Mck1 (initially called Ypk1, for yeast protein kinase) is a protein kinase homolog that cofractionates with serine, threonine, and tyrosine kinase activity (18).

MER1. *MER1* (meiotic recombination) was identified through a mutation causing production of inviable spores (22), as do many recombination-defective mutations. A *mer1* null mutation blocks meiotic recombination. Mer1 is required for splicing of *MER2* RNA (24). Mer1 has a motif found in several ribonucleoprotein-associated proteins (63).

RES1. *RES1* (Rme1 escape) was discovered through a partially dominant mutation, *RES1-1*, that permits expression of *spr3-lacZ* and sporulation of **a**/α cells expressing Rme1 (44).

RIM1,8,9,13. Recessive mutations in these genes reduce *IME1* and *IME2* expression and cause slow, inefficient sporulation (99). These mutations have pleiotropic effects on colony morphology and on growth at low temperature. Rim1 is a zinc finger protein homolog (100); the other genes have not yet been cloned.

RIM11. *RIM11* was identified through mutations that prevent expression of an *ime2-lacZ* fusion (99) and through mutations that permit survival of haploid cells genetically programmed to sporulate (i.e., expressing *IME1* constitutively) (66). Recessive *rim11* mutations prevent sporulation and meiotic gene expression. Rim11 is a protein kinase homolog (4).

RME1. *RME1* (regulator of meiosis) was discovered through an allelic difference among laboratory strains: some have a recessive *rme1* mutation that permits non-**a**/α diploids to sporulate (46). *RME1* was also initially called *CSP1* (control of sporulation) (39). An *rme1* disruption permits *IME1* expression and sporulation in non-**a**/α cells; *rme1* mutations do not alter the nutritional requirements for sporulation (68). Overexpression of *RME1* blocks *IME1* expression and sporulation (15, 68). Rme1 is a zinc finger protein homolog (15).

RPD3. *rpd3* (reduced potassium dependency) mutations permit expression of *spo13-lacZ* in vegetative, non-**a**/α cells (110), permit *ime2-HIS3* expression in Δ*ime1* strains (3), and alter the regulation of many nonmeiotic genes (110). Rpd3 has no informative homologies (110).

SIN3. *sin3* (switch-independent) mutations permit expression of *spo13-lacZ* and several early meiotic genes in vegetative, non-**a**/α cells (97), permit *ime2-HIS3* expression in Δ*ime1* strains (3), and alter the regulation of many nonmeiotic genes (5, 96, 111). *SIN3* has also been called *RPD1* (111), *SDI1* (5), *UME4* (97), and *GAM2* (119). A *sin3* null mutation reduces sporulation efficiency (111). Sin3 is a nuclear protein with four putative paired amphipathic helices (112).

SME2. A multicopy *SME2* (start of meiosis) plasmid permits sporulation in the presence of ammonia or glucose (47). An *sme2* disruption mutation does not affect sporulation. Increased *SME2* dosage may stimulate the expression of one or more late meiotic genes specifically (47).

SME3. A multicopy *SME3* (start of meiosis) plasmid permits sporulation in the presence of ammonia or glucose and in non-**a**/α cells (47). An *sme3* disruption mutation does not affect sporulation.

SPS1. *SPS1* was identified as a middle sporulation-specific transcript (73). *sps1* mutations block sporulation after the meiotic divisions (74) and cause reduced expression of late meiotic genes (85). Sps1 is a protein kinase homolog (85).

UME1,2,3,5. Mutations in these genes permit the expression of several early meiotic genes in vegetative, non-**a**/α cells (97).

UME6. *ume6* loss-of-function mutations permit *spo13-lacZ* expression in vegetative, non-**a**/α cells (97) and permit *ime2-HIS3* expression in Δ*ime1* strains (3). *UME6* was independently identified as *CAR80* from its role in expression of the nonmeiotic gene *CAR1* (72). *ume6* null mutations reduce sporulation efficiency and spore viability and cause slow growth (3). A different type of allele, originally called *rim16-12*, was identified as a mutation that permits survival of haploid cells genetically programmed to sporulate (4, 66). *rim16-12* causes reduced sporulation and *ime2-lacZ* expression but does not affect spore viability or growth.

A number of mutations that may affect meiotic RNA levels have not been characterized in great detail. *spo17* and *spo18*

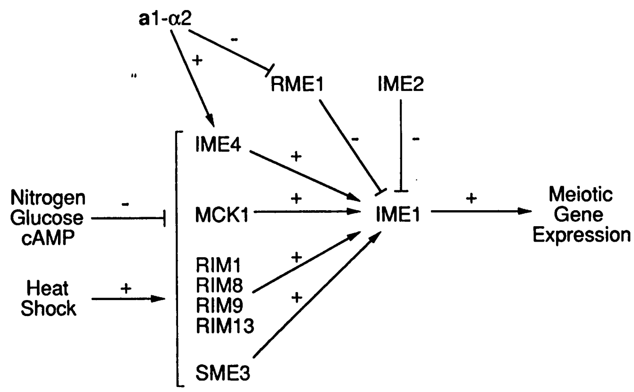

FIG. 2. Regulators that govern *IME1* expression. Regulatory relationships between global signals, regulatory genes, and *IME1* RNA accumulation are diagrammed. +, positive effect; −, negative effect specifically on transcript accumulation or promoter activity for the next gene in the series. The genes *IME4*, *MCK1*, and *RIM1* have additional roles in sporulation that are independent of *IME1* expression. The natural role of *SME3* is uncertain, as explained in the text. Expression of *IME4* and *SME3* increases in response to nitrogen limitation and growth limitation, respectively; which signals the other genes transmit is uncertain. Expression of *RME1* increases in response to nitrogen limitation in non-**a**/α cells (not diagrammed). *IME2*, an early meiotic gene, is required for downregulation of *IME1*.

mutations were identified through defects in sporulation-specific glucoamylase activity (95). There is also a large collection of *spoT* mutations (107), which block sporulation at various steps.

REGULATORY PATHWAYS

The regulation of meiotic genes may be summarized in broad outline very simply. Starvation of **a**/α cells causes increased expression of *IME1*; Ime1 then stimulates the expression of many meiotic genes. With this view in mind, I will first examine how these signals govern *IME1* expression and then how Ime1 activates meiotic genes. Subsequent sections expand and qualify this simplified view.

Control of *IME1* by the Mating Type Locus

Meiotic gene expression and sporulation depend on the repressor **a**1-α2, which determines the **a**/α cell type. How does a repressor permit cells to enter meiosis? One mechanism involves an inhibitor of meiosis specified by *RME1* (Fig. 2). In non-**a**/α cells, Rme1 blocks meiosis, as indicated by the observation that *rme1* loss-of-function mutations permit non-**a**/α cells to sporulate (46, 68, 79). In **a**/α cells, *RME1* is transcriptionally repressed through an upstream **a**1-α2 binding site (15, 68). Thus, *RME1* RNA is present at 10- to 20-fold-higher levels in vegetative non-**a**/α cells than in vegetative **a**/α cells (68). *RME1* RNA levels increase a further 10-fold in non-**a**/α cells after starvation, so a 100-fold difference in *RME1* RNA levels determines the ability or inability to sporulate (17). Expression of *RME1* in **a**/α cells, through increased gene dosage or by fusion to a constitutive promoter, reduces sporulation efficiency and meiotic gene expression (15, 32, 68). Therefore, Rme1 can inhibit sporulation, regardless of cell type, if it is expressed.

Two observations had suggested that repression of *RME1* was not the only mechanism by which **a**1-α2 stimulates meiosis. First, in comparisons of strains that lack *RME1* function, **a**/α diploids sporulate more efficiently than non-**a**/α diploids (68). Second, expression of *RME1* blocks sporulation more efficiently in non-**a**/α cells than in **a**/α cells (93). More direct evidence for an *RME1*-independent pathway that influences meiosis came from identification of the *RES1* and *IME4* genes. *RES1* was identified through a partially dominant mutation, *RES1-1*, that permits expression of a sporulation-specific *spr3-lacZ* fusion in **a**/α cells carrying a multicopy *RME1* plasmid (44). *RES1-1* also permits sporulation of non-**a**/α diploids. Two findings suggest that *RES1-1* acts through a different pathway from *RME1*. First, *RES1-1* permits higher levels of sporulation than an *rme1* null mutation in non-**a**/α diploids. Second, *RES1-1* and *rme1* mutations have additive effects on the sporulation of non-**a**/α diploids (44). These independence arguments should be considered provisional, however, because the nature of the *RES1-1* alteration (loss or gain of function) is unclear.

An attempt to clone *RES1* led to identification of a suppressor, *IME4*, that specifies a positive regulator of meiosis (86). Increased *IME4* dosage permits non-**a**/α diploids to sporulate; an *ime4* insertion mutation blocks sporulation. (In some strains, *ime4* mutations have little effect on sporulation, as discussed below.) *IME4* expression is meiosis specific: RNA levels are low in vegetative cells and increase in response to nitrogen starvation only in **a**/α cells. Because **a**1-α2 is known to act only as a repressor, it was proposed that **a**1-α2 stimulates *IME4* expression indirectly, for example, by repressing a repressor of *IME4* (86). (The recent observation that insertions lying 3′ to *IME4* lead to cell type-independent *IME4* expression suggests that a more unusual regulatory mechanism may be involved [11].) These expression and dosage studies indicate that *IME4* transmits an **a**/α cell type signal.

What is the relationship between Rme1 and Ime4? An *rme1* mutation does not alter regulation of *IME4* expression by **a**1-α2, so Rme1 is not the hypothetical repressor of *IME4* (86). An *rme1* mutation can suppress an *ime4* insertion mutation to permit expression of the meiotic genes *IME1* and *IME2*. Thus, in principle, Rme1 may act either in parallel to or downstream of Ime4. Given that **a**1-α2 represses *RME1* expression directly (15), the simplest explanation is that Rme1 and Ime4 act in parallel pathways (86).

The ultimate target of regulation by Rme1 and Ime4 is expression of the *IME1* gene. *IME1* is expressed at low levels in vegetative cells and at 5- to 30-fold-higher levels in starved **a**/α cells (45, 92). Ime1 is formally a positive regulator of other meiotic genes, because deletion of *IME1* prevents the expression of other early (*SPO11*, *SPO13*, *MER1*, *IME2*, and *HOP1*), middle (*SPS1* and *SPS2*), and late (*SGA1*) meiotic genes (23, 48, 67, 109). The **a**/α cell type regulatory signal is transmitted by *IME1* RNA levels, because expression of *IME1* from a cell type-independent promoter permits expression of meiotic genes regardless of cell type (94). Therefore, Ime1 plays a pivotal role in the activation of early meiotic genes.

Ime4 is required to stimulate *IME1* expression, because an *ime4* insertion mutation blocks *IME1* RNA accumulation in starved **a**/α cells (86). However, suppression studies indicate that Ime4 may have an additional role in stimulating meiosis, although two experiments gave apparently conflicting results. In one experiment, an *RES1-1* mutation permits high levels of *IME1* RNA accumulation in an *ime4* mutant but permits only inefficient sporulation. On the other hand, the presence of an *IME1* multicopy plasmid in an *ime4* mutant permits efficient sporulation. One idea that reconciles these observations is that Ime1 and Ime4 have partially overlapping functions: overexpression of Ime1 from a multicopy plasmid would alleviate the need for Ime4, but expression of Ime1 at more normal levels (in the *RES1-1 ime4* mutant) would not (86). In addition, differences in the translation of *IME1* RNA in these two situations (87) might account for these results.

IME4 RNA levels respond to both cell type and nutritional signals, as described above (86). Thus, increased *IME4* expression may lead directly to increased *IME1* expression in nitrogen-starved **a**/α cells. However, a second nitrogen regulatory pathway must exist, because *IME1* is regulated by nitrogen in an *ime4 RES1-1* double mutant (86).

Three lines of evidence indicate that *IME1* is the target of repression by Rme1. First, *rme1* loss-of-function mutations permit *IME1* expression in starved non-**a**/α cells (45, 92). Second, expression of *RME1* (from a constitutive promoter) in **a**/α cells prevents *IME1* expression (15). Third, expression of *IME1* from a constitutive promoter overrides the inhibition of meiotic gene expression and sporulation by Rme1 (15). These observations argue that Rme1 blocks *IME1* expression in non-**a**/α cells. In addition, *IME1* may be the only gene required for meiosis that is repressed by Rme1.

Rme1 acts over a considerable distance to repress *IME1*. Early studies suggested that Rme1 might act through a site 3 kb upstream of *IME1*, because a multicopy plasmid carrying this region could apparently titrate Rme1 activity (32). More recently, deletion analysis has indicated that a 500-bp interval that lies 2 kb upstream of *IME1* is required for repression by Rme1 (16). Mobility shift experiments indicate that Rme1, a zinc finger protein, binds to a site in this interval. Oddly, a region containing the Rme1 binding site has the properties of an Rme1-dependent activation sequence when separated from flanking DNA (16). Repression of either *IME1* or a heterologous promoter (in artificial constructs) requires the Rme1 binding site together with the adjacent 300-bp interval. Deletion of the Rme1 binding site from the chromosome does not fully relieve Rme1-dependent repression, so there may be other functional Rme1 binding sites (such as the putative site 3 kb upstream). These aspects of Rme1-dependent repression—action over a large distance and dependence on multiple sequence elements—are similar to the properties of the silencers that repress silent mating type information at *HML* and *HMR* (51). Whether these analogies reflect more fundamental mechanistic similarities between Rme1-dependent repression and silencing remains to be determined.

Other Regulators of *IME1* RNA Levels

The regulation of *IME1* RNA levels is complex but can be considered as three phenomena. First, there is a low, basal *IME1* RNA level in growing cells. This level is similar in both **a**/α and non-**a**/α cells and is reduced by glucose (45). Second, there are elevated *IME1* RNA levels under some circumstances not associated with sporulation; these include heat shock (87) and the end of exponential growth (47). Neither of these responses has been compared in **a**/α and non-**a**/α cells. Third, there is the high *IME1* RNA level observed after nitrogen starvation of **a**/α cells, which is associated with meiosis (45). This level is 5- to 30-fold higher than the basal level (45, 92); it is possible that differences in the synchrony of the sporulating population account for the differences in maximal RNA levels detected. cAMP depletion can bypass the need for nitrogen starvation to stimulate *IME1* expression (and sporulation), but this effect may be an indirect consequence of growth arrest or cell cycle arrest (62, 92). One study argues that mitochondrial function is required for maximal *IME1* RNA accumulation (106), but the possibility that energy depletion simply prevented all RNA synthesis was not ruled out. The genes described in this section influence the decision to enter meiosis through effects on *IME1* RNA levels, but which of the many possible signals they transmit is unclear.

MCK1, which specifies a putative Ser-Thr-Tyr protein kinase, is expressed at a constant level independent of cell type, glucose, or nitrogen (70). *mck1* null mutations reduce sporulation efficiency, cause accumulation of immature asci, and also cause an array of phenotypes that reflect defective mitotic centromere behavior (70, 88). The pleiotropic effects of *mck1* mutations raised the question of whether its partial sporulation defect simply reflected general ill health or whether Mck1 was required more directly for *IME1* expression. Three observations suggest a more direct role for Mck1 (70). First, overexpression of *MCK1* increases *IME1* expression in starved **a**/α cells and accelerates sporulation. This finding suggests that Mck1 activity is normally limiting for *IME1* expression. Second, *mck1* mutations cause defects in the basal level of *IME1* promoter activity in vegetative cells, as assayed with an *ime1-HIS3* fusion gene in which the *IME1* promoter is fused to the *HIS3* coding region. Under these growth conditions, the *mck1* mutant displayed no obvious growth or mitotic chromosome segregation defects (88). Third, the slow and inefficient sporulation of *mck1* null mutants is suppressed by expression of *IME1* from the *GAL1* promoter (causing fivefold *IME1* overexpression) or *ACT1* promoter (causing *IME1* expression at roughly the wild-type level) (70, 99). This finding indicates that reduced *IME1* expression may be the sole cause of inefficient sporulation in the mutant. However, the ascus maturation defect of *mck1* mutants is not suppressed by artificially elevated *IME1* expression. Together, these observations argue that Mck1 functions independently to stimulate *IME1* expression, ascus maturation, and mitotic centromere behavior.

The *RIM1*, *RIM8*, *RIM9*, and *RIM13* genes are also required for *IME1* RNA accumulation (99). Mutations in any of these genes lead to reduced *IME1* expression in meiotic cells, reduced meiotic gene expression, and slow sporulation. Because the *rim* mutations are recessive, they are inferred to result in loss of gene function, but this inference is only known to be true for *rim1* mutations (100). Like *mck1* mutations, *rim1/8/9/13* mutations are suppressed by expression of *IME1* from the *ACT1* promoter and cause reduced *ime1-HIS3* expression in vegetative cells. However, these *RIM* gene products appear to act independently of Mck1, because all *rim mck1* double mutants display more severe meiotic gene expression and sporulation defects than the single mutants (99). In contrast, double mutants with two *rim* mutations are no more defective than *rim* single mutants. Support for a close functional relationship among the *RIM1/8/9/13* gene products comes from their shared pleiotropic mutant phenotypes, including smooth colony morphology (in the otherwise rough SK-1 genetic background) and cold-sensitive growth.

mck1 and *rim1/8/9/13* mutations do not affect *IME1* expression indirectly through effects on *RME1* expression. *mck1 rim* double mutants are defective in sporulation in *rme1* deletion strains, and *MCK1* and *RIM1* are required for the activity of an *IME1* promoter fragment that is not repressed by Rme1 (54, 70, 99). Therefore, *RME1*, *MCK1*, and *RIM1/8/9/13* govern *IME1* expression independently.

mck1 and *rim1/8/9/13* mutations do not act through effects on *IME4* expression, either. The evidence comes from a strain difference: *ime4* mutations cause a complete sporulation defect in S288C-derived yeast strains (86) but cause only a marginal sporulation defect in SK-1-derived yeast strains (100). In SK-1 strains, *mck1* and *rim1/8/9/13* mutations cause more severe sporulation defects than *ime4* mutations. Therefore, *MCK1* and *RIM1/8/9/13* cannot simply be required for *IME4* expression. In addition, *ime4 mck1* and *ime4 rim1* double mutants have more severe sporulation defects than the single mutants (100). These observations suggest that *MCK1*, *RIM1/8/9/13*, and *IME4* all define independent pathways that stimulate *IME1* expression.

The cAMP synthesis and response pathway also influences sporulation, in part, through effects on *IME1* expression. Mutations that diminish cAMP-dependent protein kinase activity lead to *IME1* expression and sporulation in the absence of nitrogen starvation (7, 62, 92). Mutations that cause elevated, constitutive protein kinase activity lead to failure to express *IME1* or to sporulate (62). Although these genetic experiments suggest that the cAMP pathway may respond or govern response to nitrogen levels, the bulk of the evidence favors a role for this pathway in glucose sensing (7, 33). An increased dosage of either of two cAMP phosphodiesterase structural genes permits *ime1-HIS3* expression in *mck1* or *rim1* null mutants (69a). Therefore, regulation of *IME1* by cAMP levels does not require Mck1 or Rim1.

The *SME3* gene has the properties of a positive regulator of *IME1*, because increased *SME3* dosage causes elevated *IME1* RNA accumulation, particularly in the presence of ammonia or glucose (47). *SME3* RNA levels are very low during exponential growth and increase dramatically as cultures reach stationary phase; the response is comparable in **a**/α and non-**a**/α cells. Thus, *SME3* might relay a signal related to glucose, nitrogen, or growth. However, an *sme3* disruption mutation has no effect on sporulation efficiency or, by inference, on *IME1* expression. These results may indicate either that *SME3* acts in one of several functionally redundant pathways or that *SME3* acquires a novel function (stimulation of *IME1* expression) only when overexpressed. The relationships between *SME3* and other regulators of *IME1* expression are unclear at present.

Why should there be such a bewildering array of regulators and pathways that govern *IME1* expression? One might argue that the sensitive genetic isolation strategies tend to magnify the effects of minor metabolic perturbations. However, it may be useful for the cell to couple *IME1* expression to the sum of several metabolic signals. Thus, the decision to sporulate—which presumably reflects a threshold Ime1 concentration (see below)—would be based on a general picture of nutrient availability. That general picture may ensure that sporulation can be initiated before the nutrient supply is completely exhausted. The large size of the *IME1* 5' regulatory region (16, 32) could certainly provide the opportunity for many regulatory proteins to act.

Functional Roles of Ime1 and Ime2

Ime1 is ultimately required for the expression of most or all of the early meiotic genes, as judged from the finding that Δ*ime1*/Δ*ime1* diploids fail to express these genes (see above). Experiments in which *IME1* is expressed constitutively (cited above) argue that *IME1* transmits the **a**/α cell type signal. Similarly, expression of *IME1* in growing cells leads to elevated accumulation of transcripts of the early meiotic genes *SPO11*, *SPO13*, *HOP1*, and *IME2* but not of the middle genes *SPS1* and *SPS2* (94). These results suggest that early genes are more direct targets of Ime1 than later genes and that *IME1* RNA levels are partly responsible for transmitting the starvation signal. Thus, an understanding of Ime1 function is a good

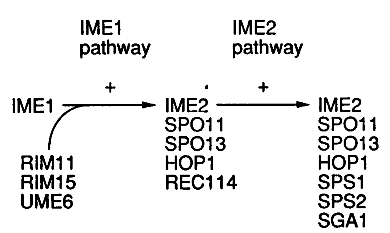

FIG. 3. Relationship between Ime1, Ime2, and early meiotic genes. Ime1 activates meiotic genes through two pathways. In the Ime1 pathway, Ime1 activates genes independently of Ime2. In the Ime2 pathway, Ime1 acts only indirectly by stimulating expression of *IME2*; Ime2 then activates genes independently of Ime1. The Ime1 pathway depends on Rim11, Rim15, and Ume6 for activation of *IME2* and, possibly, other early meiotic genes. The Ime2 pathway is independent of Rim11 and permits Ime2 to activate its own expression. The symbols in this diagram are the same as for Fig. 2.

starting point for understanding how meiotic genes are regulated.

Ime1 activates meiotic genes through two genetically distinct pathways: one is independent of the *IME2* gene product, and the other is dependent upon *IME2* (67). I will refer to these pathways as the Ime1 pathway and the Ime2 pathway, respectively (Fig. 3). In wild-type cells, Ime1 is required for both pathways because Ime1 is required for *IME2* expression (92, 118). Either pathway can stimulate RNA accumulation from many of the same early meiotic genes, including *SPO11*, *SPO13*, *HOP1*, and *IME2* itself. Each pathway has unique properties as well, so that coordination of the pathways is critical for efficient sporulation.

Evidence for the Ime2 pathway comes from experiments in which *IME2* was expressed from a hybrid *GAL1-IME2* 5' region (94). Expression of the *GAL1-IME2* hybrid gene depends on the galactose regulatory system rather than on Ime1, so that the consequences of Ime2 activity in the absence of Ime1 can be examined directly. A control Δ*ime1*/Δ*ime1 IME2*/*IME2* diploid failed to express the early genes *SPO11*, *SPO13*, and *HOP1* as well as the middle genes *SPS1* and *SPS2*. However, the Δ*ime1*/Δ*ime1 GAL1-IME2*/*GAL1-IME2* diploid expressed all of those genes. For reasons that are unclear, nitrogen starvation was required for expression of the *GAL1-IME2* gene and, consequently, for expression of the early and middle genes. However, these findings clearly indicate that Ime2 can stimulate the expression of several meiotic genes in the absence of Ime1.

Functional expression of meiotic genes through the Ime2 pathway was verified by the ability of Δ*ime1*/Δ*ime1 GAL1-IME2*/*GAL1-IME2* diploids to undergo meiotic levels of gene conversion at the *HIS4* locus and to sporulate (94). However, sporulation of these diploids is aberrant: sporulation is asynchronous and inefficient, spore viability is low, and the frequency of chromosome III disomy among spores is high. These phenotypes are not simply a consequence of Ime2 overexpression, because *IME1*/*IME1 GAL1-IME2*/*GAL1-IME2* diploids sporulate with fidelity. These observations argue that Ime1 has some unique role in sporulation that Ime2 cannot carry out. In fact, the early meiotic gene *REC114*, which is required for recombination, is activated through the Ime1 pathway but not through the Ime2 pathway (76). (It has been suggested that the Ime2 pathway also cannot activate *HOP1* expression [109], but *HOP1* expression in an *ime1* mutant that expresses *IME2* was not examined. *HOP1* is expressed in a Δ*ime1 GAL1-IME2* strain [93].) Because recombination defects lead to aneuploidy and spore inviability, it is possible that failure to express *REC114* is responsible for sporulation defects when only the Ime2 pathway is active.

Evidence that the Ime1 pathway stimulates early meiotic genes independently of Ime2 comes from examination of *IME1/IME1* Δ*ime2*/Δ*ime2* strains (94). These strains express early genes efficiently in response to starvation. Thus, Ime1 can stimulate the expression of several meiotic genes in the absence of Ime2.

Functional expression of meiotic genes through the Ime1 pathway is supported by the finding that *ime2* null mutations do not block meiotic gene conversion (94), although the rate of conversion is slowed. However, *ime2* null mutants fail to sporulate (92, 118). Thus, Ime2 must have a unique role in sporulation that Ime1 cannot carry out. Some evidence suggests that *ime2* null mutants express middle and late meiotic genes poorly (48, 94), so one unique role for Ime2 may be to stimulate later meiotic gene expression. Ime2 is also required to downregulate *IME1* RNA levels (92). In wild-type strains, *IME1* is expressed only for a brief period of time: *IME1* RNA levels are maximal 4 h after starvation (in SK-1 strains) and decline at 6 to 8 h. In *ime2* null mutants, *IME1* RNA levels do not decline until 20 to 30 h. Therefore, Ime2 is formally a negative regulator of *IME1* expression. Prolonged expression of *IME1* in *ime2* mutants probably accounts for their prolonged expression of early meiotic genes (94). The extended expression of *IME1* and early meiotic genes may interfere with the progress of *ime2* mutants through sporulation.

Ime1 and Ime2 are not homologous and thus may activate meiotic genes through different mechanisms. Ime2 is a serine/threonine protein kinase homolog (118); it has autophosphorylation activity in immune complexes (93). It has been suggested that Ime2 might stimulate meiotic genes by inactivating one of the negative regulators Ume1/2/3/5 or Sin3 (97). Thus far, direct phosphorylation of a regulatory protein by Ime2 has not been demonstrated.

Genetic evidence indicates that Ime1 may activate some meiotic genes by providing a transcriptional activation domain (91). The argument is based on studies of transcriptional activation by a *lexA-IME1* fusion-encoded protein, in which the LexA DNA-binding domain is fused to Ime1. Transcriptional activation by LexA-Ime1 was assayed through expression of a *gal1-lacZ* reporter gene with upstream *lexA* operators in place of the *GAL1* UAS. There are three correlations between the requirements for activation by LexA-Ime1 and for natural Ime1 activity, as assayed through expression of an *ime2-lacZ* reporter gene. First, four *ime1* missense mutations reduce both LexA-Ime1 and Ime1 activities, and intragenic suppressors of two mutations restore both activities. These mutations do not simply reduce accumulation of LexA-Ime1, so they seem to affect intrinsic activity. Second, *rim11* mutations block both LexA-Ime1 and Ime1 activities. Third, the central tyrosine-rich region of Ime1, which has the functional properties of an activation domain, can be replaced by the acidic herpesvirus VP16 activation domain to restore Ime1 function. Consistent with the idea that Ime1 functions directly in transcriptional activation is the finding that an Ime1–β-galactosidase fusion protein is concentrated in the nucleus. Although there is no evidence that Ime1 binds directly to DNA, the studies described below (3) suggest that a protein may act as an adaptor to permit Ime1 to bind to DNA.

The Ime1 pathway and Ime2 pathway stimulate early meiotic genes through different sites or combinations of sites. A minimal UAS from *IME2* (T_4C site and URS1) is activated by the Ime1 pathway and not by the Ime2 pathway (3). The *REC114* promoter, which includes UAS_H and URS1 sites, is also activated only through the Ime1 pathway (76). Thus, the URS1-T_4C and URS1-UAS_H site combinations may be activated by the Ime1 pathway, whereas an unidentified site (or combination of sites) may be activated by the Ime2 pathway.

Why does a cell need both Ime1 and Ime2 to turn on many of the same genes? Ime2 is a positive regulator of its own expression (3). Therefore, if a cell makes enough Ime1 to activate *IME2* expression, Ime2 can amplify Ime1 activity. This arrangement is ideal for converting a graded signal that responds to multiple inputs (*IME1* RNA levels) into a qualitative decision to activate meiotic genes and enter meiosis. In addition, amplification of Ime1 activity by Ime2 may ensure balanced expression of the many early genes required for successful recombination and segregation.

Other Positive Regulators of Early Meiotic Genes

The *RIM11* and *RIM15* gene products are required in addition to Ime1 for *IME2* expression (66, 99). *RIM11* is clearly a positive regulator of *IME2*, as determined by studies of bona fide null mutants (4). *RIM15* has been identified by a single recessive mutation, so its assignment as a positive regulator of *IME2* is tentative. *rim11* and *rim15* mutations block the activity of an *IME2* UAS that responds only to the Ime1 pathway (3), indicating that Rim11 and Rim15 act in the Ime1 pathway. These gene products act in parallel or downstream of Ime1, because they are required for *IME2* UAS activity even when *IME1* is expressed from the *GAL1* promoter (3). Rim11 is required only for the Ime1 pathway, because expression of the *GAL1-IME2* hybrid gene activates the *IME2* promoter and permits sporulation in a *rim11* mutant (66). Whether Rim15 is also specific for the Ime1 pathway is unclear.

Rim11 appears to be more directly required for Ime1 activity than for some other aspect of *IME2* UAS activity. This idea comes from the observation that a *rim11* mutation blocks transcriptional activation by the LexA-Ime1 fusion protein (91). The *rim15* mutation has little effect on LexA-Ime1 activity, so it seems unlikely that *RIM11* is simply required for *RIM15* expression or activity. *RIM11* specifies a serine-threonine protein kinase, as determined by sequence analysis and immune complex phosphorylation assays (4). There is no evidence at present that Ime1 is phosphorylated, so details of the molecular interactions between Ime1 and Rim11 are unknown.

The *rim16-12* mutation also blocks *IME2* UAS activity without affecting Ime1 polypeptide levels (3, 66). Recent studies indicate that *rim16-12* is an unusual allele of *UME6* (4), which is discussed below.

Negative Regulators of Early Genes

Two negative regulators, Sin3 and Rpd3, contribute to the proper expression of many early meiotic genes. Null *sin3* and *rpd3* alleles permit elevated expression of a *spo13-lacZ* fusion in vegetative, non-**a**/α cells, so Sin3 and Rpd3 are negative regulators of *SPO13* (97, 110). *sin3* mutations, which have been characterized more extensively, permit the expression of other early meiotic genes (*SPO11*, *SPO16*, and *IME2*) but not later genes (*SPO12* and *SPS2*) in vegetative, non-**a**/α cells (97). *sin3* and *rpd3* mutations cause increased expression of a number of nonmeiotic genes, such as *HO*, *TRK2*, *STE6*, and *RME1*, under inappropriate conditions. More detailed studies indicate that Sin3 and Rpd3 are required for the full range of expression of many regulated genes; that is, *sin3* and *rpd3* mutations cause elevated expression under repressing or noninducing conditions and cause reduced expression under derepressing or inducing conditions (110, 111). Thus, Sin3 and Rpd3 are referred to as transcriptional modulators. Existing evidence indicates only a negative role for Sin3 and Rpd3 in early meiotic gene expression.

What is the relationship between Sin3, Rpd3, and the positive regulators of early meiotic genes? Sin3 and Rpd3 seem to act in the same pathway, because *sin3 rpd3* double mutants express *spo13-lacZ* during vegetative growth at the same level as either single mutant (110). Relationships with Ime1 and Ime2 have been studied in greater detail with Sin3 than Rpd3. *spo13-lacZ* is expressed in *sin3* mutants, *sin3 ime1* double mutants, and *sin3 ime2* double mutants (97). Therefore, Sin3 may act downstream or independently of Ime1 and Ime2. Expression of Ime1 in vegetative, non-**a**/α cells causes expression only of early meiotic genes (94), just as a *sin3* mutation does, supporting a close functional relationship between Ime1 and Sin3. The sites of action of Ime1 and Sin3 are close or the same, because a *sin3* null allele permits activity of a 48-bp *IME2* UAS that responds only to the Ime1 pathway (3). However, *IME2* UAS activity increases in response to *IME1* expression in a *sin3* null mutant. Therefore, Ime1 does not stimulate a UAS simply by inactivating Sin3. The observation that an *IME2* UAS fragment can be activated by a *sin3* null mutation but not by the Ime2 pathway suggests that Ime2 does not simply inactivate Sin3. Thus, it seems likely that Sin3 acts independently of Ime1 and Ime2.

Two observations suggest that Sin3 may act directly as a negative transcriptional regulator. First, Sin3 is concentrated in the nucleus (112). Second, a LexA-Sin3 hybrid protein can block the activation of a reporter gene that contains LexA binding sites (113). The region of Sin3 required for repression by LexA-Sin3 is also required for negative regulation of natural Sin3 target genes. There is no evidence thus far for direct binding of Sin3 to DNA, so Sin3 may exert repression by interacting with a DNA-protein complex.

Recessive mutations in *UME1*, *UME2*, *UME3*, and *UME5* cause low-level accumulation of early meiotic RNAs in nonmeiotic cells (97). Accordingly, these genes specify putative negative regulators of early meiotic genes. Detailed characterization of these genes has not been reported.

UME6, a Positive and Negative Regulator of Early Genes

The *UME6* gene product has both positive and negative effects on early meiotic gene expression. The gene was first identified as a negative regulator: *ume6* mutations permit *spo13-lacZ* expression in vegetative non-**a**/α cells (97) and permit *IME2* promoter activity, assayed by a fusion to the *HIS3* coding region (*ime2-HIS3*), in *ime1* null mutants (3). *UME6* is the same gene as *CAR80*, which was identified as a negative regulator of the arginine catabolic gene *CAR1* (72). The connection between *CAR1* and early meiotic genes is the URS1 site: URS1 was first discovered as a negative regulatory site in the *CAR1* upstream region (102). In fact, a *ume6* insertion mutation abolishes repression through URS1 in nonmeiotic cells (72). These findings indicate that *UME6* is a negative regulator of meiotic and nonmeiotic genes that acts through URS1.

One might imagine that a Ume6-dependent repression system would compete with an Ime1-dependent activation system at the URS1 sites of early meiotic promoters. This model predicts that *ume6* loss-of-function mutations should not interfere with Ime1-dependent activation. In fact, *ume6* mutants that express Ime1 might even overexpress meiotic genes. These predictions were not upheld in studies of the

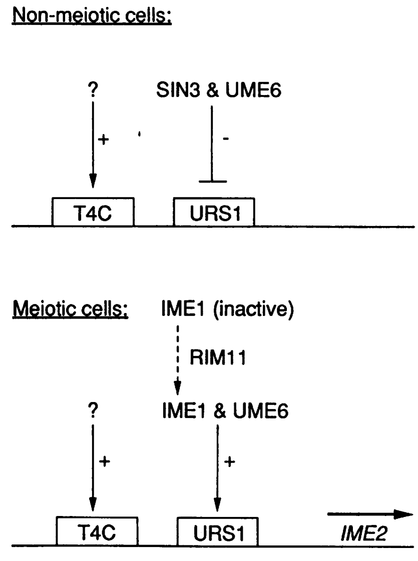

FIG. 4. Relationship between regulatory proteins and target sites at the *IME2* upstream region. In nonmeiotic cells, the *IME2* UAS is inactive because of Sin3- and Ume6-dependent repression. Repression by Sin3 and Ume6 is exerted at the URS1 site. In meiotic cells, Ime1 accumulates and is activated in a Rim11-dependent step. Active Ime1 and Ume6 together stimulate *IME2* UAS activity. Activation depends on the URS1 site and does not require Sin3. Activation also requires a T_4C site, although which regulators act there is uncertain.

IME2 UAS (3). Instead, *ume6* mutations that abolish repression also abolish Ime1-dependent activation. These experiments were conducted with a UAS that responds only to the Ime1 pathway and in *ime2* mutants, indicating that Ume6 is required for activation through the Ime1 pathway. As determined by immunoblots, Ume6 is not required for Ime1 polypeptide accumulation. Therefore, Ume6 acts in conjunction with or downstream of Ime1 to activate the *IME2* UAS. There is no evidence thus far that Ume6 is required for the activation of any other early meiotic genes.

UME6 was also identified through a mutation that may specifically impair its positive role in *IME2* expression. The *rim16-12* mutation was identified as a mutation that prevents *IME2* expression (66). Linkage and complementation analysis indicates that *rim16-12* is a *ume6* mutation (4). A *rim16-12* mutant complements a *ume6* insertion mutant for pleiotropic growth defects and spore inviability, indicating that *rim16-12* is not a null allele. *rim16-12* causes a lower level of *ime2-HIS3* expression in vegetative cells than *ume6* null mutations, suggesting that repression of the *IME2* promoter by Ume6 is intact. Therefore, *rim16-12* may cause a specific defect in activation of *IME2* and other meiotic genes. Alternatively, *rim16-12* may cause the formation of a superrepressor that reduces the expression of all Ume6-repressible genes.

Model for Ime1-Dependent Activation of Early Meiotic Genes

The observations recounted above are consistent with a simple model for the roles of Ume6 and Ime1 in regulation of the *IME2* UAS and, possibly, other early meiotic promoters as well (3). In cells that lack Ime1, Ume6 is required for the activity of a repressor that acts through the URS1 site. Ime1 then modifies the repressor to convert it into a positive regulator (Fig. 4).

What is the repressor? The genetic studies described above implicate Ume6 and Sin3 (and probably Rpd3) in repression (3, 97, 113). However, a *sin3* mutation does not block repression of the nonmeiotic *CYC1* promoter by URS1 (72). Thus, Sin3 may be required for the repression of only a subset of URS1-containing promoters. This subset may be defined by a nearby sequence or by the nature of the activator protein. Given that Ume6 is required for both repression and activation through URS1, the simplest explanation is that Ume6 binds to URS1. However, no observations support that idea at present. The major URS1-binding protein, a heterodimer called BUF (55), is present in *ume6* mutant extracts (72). Thus, BUF and Ume6 may associate or modify one another to generate the repressor.

How might Ime1 modify the repressor? The properties of LexA-Ime1 fusion proteins suggest that the role of Ime1 may be to provide a transcriptional activation domain (91). One simple possibility is that Ime1 binds directly to the URS1-repressor complex and, through the presence of an activation domain, converts the negative regulator to a positive regulator. Binding by Ime1 may be facilitated by proteins at a nearby UAS_H or T_4C site.

It is tempting to use this model to explain the regulation of all early meiotic genes. However, recall that some early meiotic regulatory regions have separable UAS regions (such as *IME2*) and others do not (such as *HOP1* and *SPO13*). This distinction may reflect more fundamental differences in mechanisms of regulation.

EFFECTS OF STARVATION ON MEIOTIC GENE EXPRESSION

The studies described above suggest that a cell with high levels of *IME1* RNA should express high levels of other early meiotic gene RNAs. However, as mentioned above, heat shock and growth limitation stimulate *IME1* RNA accumulation, yet *IME2* expression and sporulation do not occur (47, 87). Sporulation might be dismissed as an indirect assay of Ime1 activity, but certainly *IME2* RNA should be present in cells that express *IME1*. The lack of correspondence between *IME1* and *IME2* RNA levels in growing cells may result from the effects of starvation on Ime1 translation (87), Ime1 activity (91), *SIN3* expression (112), and meiotic RNA stability (see next section).

The idea that *IME1* RNA translation is regulated derives from a comparison of *IME1* RNA levels and accumulation of an *ime1-lacZ* fusion protein (87). Starvation of **a**/α cells caused a 9-fold increase in *IME1* RNA levels but a >3,000-fold increase in β-galactosidase activity. The observation was slightly complicated because the amounts of native *IME1* RNA and plasmid-encoded *ime1-lacZ* RNAs were not distinguished. However, it was observed that *IME1* has a long (220- to 280-base) untranslated leader with the potential to form a stem-loop structure. These findings have led to the suggestion that, in growing cells, the stem-loop structure blocks *IME1* translation; in starved cells, inhibition of *IME1* translation is bypassed (87).

Other effects of starvation are independent of *IME1* translation. Expression of *IME1* from the *GAL1* promoter permits a comparison of growing and starved cells with essentially the same levels of *GAL1-IME1* RNA (94) and protein (93). Although the growing cells expressed higher levels of early meiotic RNAs than wild-type cells, starvation caused a further 3- to 10-fold increase in early meiotic gene RNA levels. Two observations may explain this increase. First, Sin3-dependent repression may be lifted in starved cells. This idea derives from the observation that *SIN3* RNA is present in growing cells but not in stationary-phase cells (112). Therefore, growth limitation may lead to decreased Sin3 levels. Second, Ime1 may be a more potent transcriptional activator in starved cells. This idea derives from the finding that the LexA-Ime1 fusion protein is a 10-fold-better activator in starved cells than in growing cells (91). The C terminus of Ime1 is required for both the starvation response and Rim11 dependence of LexA-Ime1 (91). Therefore, Rim11 may relay a starvation signal.

INSTABILITY OF EARLY MEIOTIC TRANSCRIPTS

Progress through the meiotic prophase can be interrupted by providing nutrients to starved cells (25). These circumstances cause cells to resume mitotic growth (see reference 37 for a more detailed discussion). The ability of cells to rapidly exit the meiotic pathway suggested that meiotic gene transcripts and gene products might be quite unstable. Indeed, providing nutrients to sporulating cells causes the transcripts of three early meiotic genes (*SPO11*, *SPO13*, and *SPO16*) to decay with half-lives of about 3 min (103). The transcripts of two later genes (*SPO12* and *SPS2*) are considerably more stable, with half-lives of 10 to 12 min. Thus, nutrient addition prevents the continued expression of meiotic genes.

The stability of *SPO13* RNA is twofold greater in acetate sporulation medium than in a similar medium containing glucose (103). This determination was made by interrupting transcription with a temperature-sensitive RNA polymerase mutant. Stability differences were observed with both the native *SPO13* gene and an *ACT1-SPO13* fusion, in which the *ACT1* promoter was fused to the *SPO13* coding region. Two control transcripts, those of the native *ACT1* gene and a *SPO13* promoter-*HIS3* fusion, had the same half-lives regardless of carbon source. Thus, conditions that favor meiosis (presence of acetate and absence of glucose) also increase *SPO13* RNA stability.

One major determinant of *SPO13* RNA instability lies within the +3 to +262 interval, as determined by deletion and substitution analysis (103). Nonsense or frameshift mutations early in *SPO13* stabilize *SPO13* RNA, as does inhibition of protein synthesis with verrucarrin A. Therefore, the translation of *SPO13* RNA leads to its rapid degradation. It is noteworthy that sporulation is associated with decreased translation rates and ribosome numbers (25). The coupling of translation and RNA degradation may ensure that meiotic RNAs are translated under these adverse circumstances before they are degraded.

MEIOSIS-SPECIFIC SPLICING

Studies of the genes *MER1* and *MER2* indicate that a group of genes may be expressed only in meiotic cells through Mer1-dependent, meiosis-specific splicing. *mer1* mutants display reduced meiotic recombination rates and, as a consequence, produce inviable spores (22). *MER1* is expressed as an early meiotic gene (23). *MER2* was identified as a multicopy genomic clone that improves meiotic gene conversion in a *mer1* null mutant (21). (*MER2* is the same gene as *REC107* [14], in which mutations were identified by their resulting recombination defect [61].) *MER2* RNA accumulates in both meiotic and nonmeiotic cells. However, a splicing reaction that removes an 80-base *MER2* intron occurs much more efficiently in meiotic cells than in nonmeiotic cells (24). In *mer1* mutants, splicing of the *MER2* intron is inefficient in both meiotic and nonmeiotic cells. Mer1 is the only meiosis-specific product required for *MER2* splicing, because expression of *MER1* from a heterologous promoter in nonmeiotic cells permits efficient *MER2* splicing.

Mer1 is not a general splicing factor, because it is expressed only in meiotic cells and is not required for cell viability (23). Mer1 is not even required for all splicing in meiotic cells, because the meiosis-specific *MEI4* transcript is spliced efficiently in a *mer1* mutant (65). The *MER2* 5′ splice junction deviates from the strict yeast consensus sequence and may require Mer1 for recognition by the splicing machinery (24). *MEI4* and other known intron-containing meiotic genes have consensus 5′ junctions (*DMC1* [2], *MEI4* [65], and *REC114* [76]), so none of these RNAs may require Mer1 for splicing. However, expression of a *MER2* cDNA does not completely suppress the spore inviability of a *mer1* mutant (24). Therefore, Mer1 may be required for splicing of an unidentified transcript as well as of *MER2*.

COORDINATION OF EARLY AND LATER TEMPORAL CLASSES

What signals establish the temporal sequence of early and later (that is, middle and late) gene expression? One factor is that later gene expression may be dependent upon early meiotic events (43). Expression of a late *spr3-lacZ* fusion increases 100-fold after starvation in a wild-type diploid. Expression increases only 2- to 10-fold in a *cdc8/cdc8* diploid, which is defective in thymidylate kinase activity and thus in DNA synthesis. DNA synthesis is required for meiotic recombination (9, 25), so the *cdc8* mutation may have many indirect effects. However, recombination-defective mutants undergo meiotic divisions and spore formation, suggesting that recombination per se is not required for the expression of later genes (25). Meiotic DNA synthesis may generate a signal that is required for the expression of later sporulation-specific genes. The idea that DNA synthesis dependence is a timing mechanism rests on the (untested) assumption that early gene expression is independent of DNA synthesis.

A second factor that distinguishes some early and later genes is *IME2* dependence. Most early genes are expressed in an *ime2* mutant, but the later genes *SPS1*, *SPS2*, and *SGA1* are not (48, 67). Given that *IME2* is an early meiotic gene, this dependence may ensure that early genes are expressed before later gene activation.

Recent results indicate that the *SPS1* gene product has a positive role in later gene expression. An *sps1* mutation leads to a reduction in late gene RNA levels (85). *sps1* mutants arrest quite late in sporulation, after the meiotic divisions (74). Thus, the defect in late gene expression cannot be an indirect consequence of a defect in meiotic DNA synthesis. Sps1 is a protein kinase homolog (85), so there must be other members of this transduction pathway. The *SPS1* gene itself is a middle gene (74), so *SPS1*-dependent genes would be silent until the sporulation program is well under way.

CONCLUDING REMARKS

It has been 10 years since the first sporulation-specific transcripts were reported. We now have a wealth of information on the general mechanisms of meiotic gene regulation, promoter structure, meiotic regulatory genes, and the formal pathways in which these regulators act. The challenges over the next few years will be to establish the biochemical mechanisms through which these regulators act and to understand how their activities are coordinated to ensure an orderly developmental program. The mechanisms that maintain the mitotic cell cycle and meiosis as alternatives also remain to be determined. Finally, it will be exciting to see whether meiotic regulatory mechanisms in budding *S. cerevisiae* are conserved in meiotic cells of other organisms.

ACKNOWLEDGMENTS

I thank Anne Galbraith, Doug Pittman, Yang Mao, Marc Cool, John Anderson, Bob Malone, and members of my laboratory for many helpful comments and permission to cite work in progress. I also thank Mary Clancy, Don Primerano, and Jackie Segall for providing unpublished information. I am grateful to Shelly Esposito for many stimulating discussions on topics of meiotic regulation.

Work in my laboratory has been supported by Public Health Service grant GM-39581 and by funds from the March of Dimes and the American Cancer Society.

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